(PRIMARY)
IMMUNE DEFICIENCIES

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immunity is nearly invisible
immunity is nearly invisible, unless defective
the jobs of clinical immunologists
-identify the defect & restore immune function
Severe Combined Immune Deficiency (SCID)

T-B-NK- (ADA)
T-B-NK+ (RAG, ARTEMIS)
T-B+NK- (γC, JAK3)
T-B+NK+ (CD3, 22q deletion)

cell-mediated defect;

humoral defect;

opportunistic infections,
• viruses
• fungus (candida, PJP)
• mycobacterium

sinopulmonary infections,
• encapsulated bacteria
Early SCID dx and BMT determines survival but how can affected infants be identified?
T Cell Receptor Excision Circles (TRECs)
TREC Dried Blood Spot Assay

1. Guthrie Card
2. 3 mm hole punched from blood spot
3. 50 ul blood/drop
4. Extract DNA
5. ~3 ul blood
6. Measure TREC s by PCR

Jennifer Puck, UCSF
California TREC screening results, year one

500,000 TREC Screens

- 99.2% normal first test
- 99.99% normal after 1 or 2 TREC tests
- 30 (60%) normal after flow cytometry

0.01% (n=50)
Immediate Positive or DNA Amplification Failure

20 abnormal:
1 in 25,000 births

- 6 Typical SCID
- 1 Leaky SCID, Omenn syndrome
- 3 Variant SCID
- 4 Syndromes with low T cells
- 6 Secondary T lymphopenia

Estimated incidence of SCID
• Pre TREC screen, 1:200,000
• Post TREC screen, 1:50,000

Jennifer Puck, UCSF
SCID screening using TRECIs is nearly universal
identifying PID patients without a screen is art

2 or more warning signs (kinda) predicts PID
sensitivity, 56%;
specificity, 16%

**10 Warning Signs of Primary Immunodeficiency**

Primary Immunodeficiency (PI) causes children and adults to have infections that come back frequently or are unusually hard to cure. 1:500 persons are affected by one of the known Primary Immunodeficiencies. If you or someone you know is affected by two or more of the following Warning Signs, speak to a physician about the possible presence of an underlying Primary Immunodeficiency.

1. Four or more new ear infections within 1 year.
2. Two or more serious sinus infections within 1 year.
3. Two or more months on antibiotics with little effect.
4. Two or more pneumonias within 1 year.
5. Failure of an infant to gain weight or grow normally.
6. Recurrent, deep skin or organ abscesses.
7. Persistent thrush in mouth or fungal infection on skin.
8. Need for intravenous antibiotics to clear infections.
9. Two or more deep-seated infections including septicemia.
10. A family history of PI.

*Courtesy of the Jeffrey Modell Foundation*
what are the barriers to MDs considering PIDs?

• immune defects are rare; severe ones are often sporadic
• infections are common in pediatrics, not just PID
• family history is hard (and potentially messy)
• maternal IgG is present for first months of life
• presentation is the result of a gene defect and exposure for disease, so presentation is delayed and variable
• there are now hundreds PID diagnosis
the diagnosis of PID patients is relies heavily on technology

Data courtesy of NHGRI
“...the greatest teachers of modern immunology: patients with immunodeficiency diseases.”

Robert A. Good M.D. PhD.

**PID**s illuminate the cellular and molecular basis of:
- immunity (candida, pneumococcus, and mycobacteria)
- immune tolerance
- cytotoxicity and cancer susceptibility
the molecular basis of candidal control at mucosal sites

Image courtesy of G. Colm
candida covers our mucocutaneous surfaces but some have more than others
candida covers our mucosal surfaces but some have more than others
MUCOCUTANEOUS SURFACE

COMMENSAL CANDIDA

EPITHELIUM

STERILE SUB-MUCOSAL TISSUE
defects in candidal detection
Gain-of-function human \( \text{STAT}1 \) mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis 2011

Deficiency of Th17 cells in hyper IgE syndrome due to mutations in \( \text{STAT}3 \) 2009

Impairment of immunity to \( \text{Candida} \) and \( \text{Mycobacterium} \) in humans with bi-allelic \( \text{RORC} \) mutations 2015

defects in \( \text{T}_H17 \) differentiation
EXTREME HYPERIMMUNOGLOBULINEMIA E AND UNDUE SUSCEPTIBILITY TO INFECTION

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From the Departments of Pediatrics and Microbiology and Immunology, the Duke University School of Medicine, Durham, North Carolina, and the Department of Pediatrics, the Medical College of Georgia, Augusta, Georgia

HYPERIMMUNOGLOBULINEMIA E

1. Patient B.S. at 8 years of age, before and after initiation of oxacillin therapy. (Reproduced by permission of Bristol Laboratories.)
A perfect candida detection and response system for your home (mucus)
the molecular basis of mycobacterial immunity
Q: what does pictured woman on the left have in common with the one on the right?
A: childhood exposure to *M. bovis* (BCG vaccine)
BCG vaccine

BCG lymphadenitis

BCGosis
extra-pulmonary mycobacterial control ONLY

Salmonella
Coccidioides
Histoplasma
Burkholderia

MSMD
Mendelian Susceptibility to Mycobacterial Disease
endogenous and pharma anti-cytokine abs
anticytokine autoantibodies (Aab)

An adult onset immunodeficiency

In East Asia M=F, in US all F and Born SE Asia

High titer Neutralizing IgG₄

90% of HIV neg Thai patients with opportunistic infections had neutralizing anti-IFNg autoantibodies

Labs otherwise normal, but may have elevated IgG

Aab NOT in pulmonary and disseminated TB

Anti-GM-CSF autoantibodies in pulmonary and meningeal cryptococcosis and nocardiosis

Treatment: antibiotics/antifungals, Rituximab

More to come! (likely by Steve)
the molecular basis of pneumococcal immunity

Image courtesy of B. Gallet
pneumococcemia, historically a common and deadly infectious disease

Figure 1: Mortality from pneumococcal bacteremia

although Th17 cells contribute to pneumococcal immunity in mice they are expendable in humans

In humans pneumococcal immunity is mediated by serum soluble proteins

complement deficient patients are susceptible to coccemia due to poor opsonization
In humans pneumococcal immunity is primarily mediated by antibodies
most PID patients with pneumococcal susceptibilities possess B-cell defects

no B cells = no antibodies

AGAMMAGLOBULINEMIA

By Col. Ogden C. Bruton, M.C., U.S.A.
Washington, D.C.

• 8 y.o. male with 19 episodes of pneumococcal sepsis
  • absent gamma peak on protein electrophoresis
  • IM IgG initiated monthly at 100mg/kg
  • It works!
• B-cell lymphopenia in XLA described 2 decades later

Bruton 1952, Pediatrics
B cells are present but germinal center responses are defective: the hyper IgM syndromes

Facchetti et al. 1995, J. Immunol

(Warnatz et al. 2006, Blood)

(Revy et al. 2000, Cell)
what can we learn about B-cell tolerance mechanisms from PID patients?

B-cell tolerance occurs at distinct developmental stages

- Pro-B cell
- Early immature B cell
- New emigrant B cell
- Mature naive B cell

- Bone marrow
- Peripheral blood
- Germinal center reaction

- V(D)J recombination
- Central tolerance checkpoint
- Peripheral tolerance checkpoint

- Non-autoreactive B cells
- Poly-reactive B cells
- Self-reactive B cells

(Adapted from Cambier, 2007 Nat. Rev. Immuno)
(Adapted from Wardemann et al., 2003, Science)
PID defects reveal the molecular players in central and peripheral B-cell tolerance

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Peripheral Blood</th>
<th>Peripheral Blood</th>
<th>Germinal Center Reaction</th>
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</thead>
<tbody>
<tr>
<td>Pro-B cells</td>
<td>Early Immature B cells</td>
<td>New Emigrant B cells</td>
<td>Mature Naïve B cells</td>
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V(D)J Recombination

Central Tolerance Checkpoint

Peripheral Tolerance Checkpoint

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>BTK</td>
<td>CD19*</td>
</tr>
<tr>
<td>CD19*</td>
<td>CD40L</td>
</tr>
<tr>
<td>MyD88</td>
<td>FOXP3</td>
</tr>
<tr>
<td>IRAK4</td>
<td>MHCII</td>
</tr>
<tr>
<td>TACI*</td>
<td>DOCK8</td>
</tr>
<tr>
<td>AID/UNG</td>
<td>TACI*</td>
</tr>
<tr>
<td>NFKB1*</td>
<td></td>
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</tbody>
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* = CVID associated genes

Courtesy of Eric Meffre
non-infectious CVID Complications are primarily autoantibody mediated

- ITP
- AIHA
- Evan’s Syndrome
- Autoimmune neutropenia
- Pernicious anemia
- Myasthenia gravis
- Thyroiditis
- Autoimmune hepatitis
- T1DM

Cunningham-Rundles et al., 2010, Blood.
the most bizarre germinal centers ever, CVID patients with autoimmune cytopenias (AICs)
like AID deficiency, CVID patients with AICs lack typical germinal center products

**serum IgA**

![Graph showing serum IgA levels in CVID-AIC and CVID+AIC patients.](image)

**class-switched memory B cells**

![Flow cytometry analysis showing IgA and IgG levels in HD, CVID-AIC, and CVID+AIC.](image)

**somatic hypermutations (SHM)**

![Graph showing mean mutation rate in VH and mutation per VH transcript.](image)

Le Coz et al, In review
somatic hypermutation mediates clonal redemption in germinal centers

Germline CDR3 (time zero):  SRSDFLLTAYDLFRKGNAMDVW
time 1  SRSDFLVTAYDLFRKGNAMDVW
time 2  SRSDFLTEYDLFRKGNAMDVW
time 3  SRSDFRYEYDLFRKGNAMDVW
time 4  SRSDFRMYEYDLFRKGYAMDVW
time 5  SRSDFRMEYDHLFRKGYAMDVW
time 6  SRSDFRMEYDHLFRKGYAVFW
time 7  SRSDFRMEYDHLFRCGYAVFW
time 8  SRSDFRMEYDHLFRCGYAVFW
time 9  SRSDFRMEYDHLFRVGYAVFW

Adapted from Sabouri et al. 2014, *PNAS*
Take home messages

PIDs may be rare diseases but they offer key insights and “proof of concept” translational opportunities for astute clinicians and scientists.

They are easy to miss, don’t do that